

Trends and Seasonal Variations in the Occurrence of *Salmonella* in Pigs, Pork and Humans in Denmark, 1995 – 2000

Trends und saisonale Abhängigkeiten beim Vorkommen von *Salmonella* in Schweinen, Schweinefleisch und Menschen in Dänemark, 1995 - 2000

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Summary:

A mandatory programme monitoring the occurrence of *Salmonella* in pork at slaughterhouses and a serological monitoring of slaughter-pig herds has been implemented in Denmark since 1993 and 1995, respectively. All results are stored in a central database. From this, aggregated weekly results of serological and bacteriological samples collected in the period between January 1995 and July 2000 were extracted. In addition, the reported weekly incidence of human infections with *S. Typhimurium* covering the same time period was obtained. The times series were analysed for trends and cyclic variations by seasonal decomposition. The association between the incidence in humans and the prevalence of *Salmonella* in pigs and pork, and prevailing weather conditions, were analysed by using a general linear (glm) and a general additive model (gam). Explanatory variables were lagged to account for time elapsed between sampling, consumption, incubation period and case registration. The results of the seasonal decomposition showed an overall declining trend in all three time series; presumably an effect of the implemented *Salmonella* control measures. All time series exhibited a double peaked annual cycle. The seasonal variation of the prevalence in pork and the human incidence had a very similar course with a starting increase in the spring and a peak in August-September. The variables that were both biologically meaningful and statistically significant in both regression models were the prevalence in pork sampled 4 to 5 weeks before case registration, the seroprevalence, measured as the average prevalence of week 15 to 35 before case registration, and the air temperature lagged at 2 and 3 weeks. Limitations on inferences from overall surveillance data are discussed.

Keywords: Control- and surveillance programmes, ambient temperature, Poisson regression, seasonal decomposition, seroprevalence

Introduction

The objective of this study was to describe the trends and seasonal variations in the occurrence of 1) *Salmonella* antibodies in slaughter pigs, 2) *S. Typhimurium* in pork, and 3) *S. Typhimurium* infections in humans by seasonal decomposition of the three time series. Furthermore, we wanted to explore the association between the number of human cases of *S. Typhimurium* and the prevalence of *Salmonella* in slaughter pigs and pork. *S. Typhimurium* was chosen as an indicator of pork-associated infection (Anonymous, 2000; Hald and Wegener, 1999). Because seasonal variation was observed in all three time series, meteorological data were also included.

Materials and methods

A mandatory programme monitoring the occurrence of *Salmonella* in pork at Danish slaughterhouses was initiated in 1993 (Bager et al., 1995). This was followed by a serological monitoring of slaughter-pig herds in 1995 (Mousing et al., 1997). Cases of human salmonellosis are reported to the Statens Serum Institut, which is the reference laboratory for enteric pathogens and in charge of the laboratory surveillance system. All monitoring data are stored in central databases at the Danish Zoonosis Centre, a network including the Danish Veterinary Laboratory, the Danish Veterinary and Food Administration, and the Statens Serum Institut. From this database, the following aggregated weekly results covering the period from the 1st week of 1995 to the 30th week of 2000 were extracted:

1. The proportion of seropositive meat-juice samples collected from slaughter pigs (PIG series). Individual samples were defined as positive, if the optical density (OD) % was above 40.
2. The proportion of samples of fresh pork positive for *S. Typhimurium* (PORK series).
3. The weekly number of registered cases of *S. Typhimurium* (HUMAN series).

From the Danish Meteorological Institute, we obtained weekly data on different climate observations covering the same period.

Seasonal time series Y_t (time unit = $t = 1$ to N) can be decomposed into trend T_t , seasonal S_t and remainder R_t components by a sequence of robust locally distance weighted regressions (loess-smoothings), where $Y_t = T_t + S_t + R_t$ (Cleveland et al., 1990). The S-plus function `stl` was applied for the decomposition of the three time series mentioned above (S-PLUS, 2000). The parameter values to be used in the

decomposition were chosen on the basis of the recommendations given by Cleveland et al. (1990).

In order to identify possible predictors for human cases of *S. Typhimurium*, two Poisson regression models were developed. The outcome variable was the number of human cases of *S. Typhimurium* and the explanatory variables were the seroprevalence in slaughter pigs, the prevalence of *S. Typhimurium* in fresh pork, and the following meteorological data: temperature in °C, hours of sunshine, and days with rain.

One of the assumptions in a Poisson regression model is that the number of cases in one week is independent of the number of cases in other weeks. This was not the case in the present study. In an attempt to deal with this, we took two approaches by using a generalised linear model (glm) and a generalised additive model (gam, where a four-parameter spline was used for smoothing). Generalised additive modelling makes it possible to account for non-linear dependencies and optimise the smoothing of the variables with respect to minimisation of the autocorrelation in the residuals (Brumback et al., 2000, Schwartz et al., 2000). In both the glm and gam, lagged series of the explanatory variables were used (Schwartz et al., 2000). Akaike's Information Criteria (AIC) was used as goodness of fit in an automated stepwise selection of variables to be included in the final models. Furthermore as a flexible seasonal baseline, the seasonal component from the decomposition of human time series was added after the stepwise selection of the lagged values. Finally, to account for overdispersion, a dispersion parameter was estimated.

Results

Decomposition of time series

The results of the seasonal decomposition of the PIG, PORK and HUMAN series all showed an overall declining trend (Figure 1). The seroprevalence in slaughter pigs was reduced from a 6 %-level to about 3 % during the first three years, but reached a steady-stage after this period. In the first three years, the trend of the prevalence in pork was rather stationary at a level of 0.5-0.7 %. From February 1998, the prevalence showed a declining trend reaching a minimum of 0.2 % at the end of the study period. According to the trend analysis, the number of weekly cases of *S. Typhimurium* was reduced from approximately 15 to 9 in the study period with the steepest decline occurring in the initial two-year period. All time series also exhibited seasonal variation that was annual in period. The seasonality in the seroprevalence was most distinct in the first part of the study period, where the seroprevalence peaked in the late winter and early fall. The seasonal variation of the prevalence in pork and the incidence in humans had a very similar course with a starting increase in the spring and a peak in August-September (Figure 2).

The late summer peak in the pork prevalence appeared 3-4 weeks before the peak in human cases.

Poisson regression models

Results from the glm showed that the weekly number of human *S. Typhimurium* cases was significantly associated with the prevalence in pork sampled 1 to 5 weeks before case registration. A significant association was also found between the seroprevalence, measured as the average prevalence 15 to 35 weeks before case registration, and the human incidence. The major meteorological variable which demonstrated statistically significant association with the human incidence was air temperature lagged at 2 and 3 weeks. Of all the positively associated variables, the air temperature and the prevalence in pork lagged at 2 weeks resulted in the greatest reduction in the deviance (Table 1).

The gam resulted in the following final model:

$\text{CASES} \sim \text{s(DWR1)} + \text{TEMP2} + \text{HOS3} + \text{s(TEMP3)} + \text{PORK4} + \text{PORK5} + \text{s(SERO)} +$
seasonal component,

where DWR1 is days with rain lagged 1 week,
HOS3 is hours of sun lagged 3 weeks,
TEMP2/3 is the air temperature lagged at 2 and 3
weeks, respectively,
PORK4/5 is the prevalence in pork lagged at 4 and 5
weeks, respectively, s() indicates that the included
variable has been smoothed, i.e. a non-linear
dependency was assumed.

The parameter estimates indicated that days with rain (DWR1) and hours of sun (HOS3) were negatively associated with the number of human cases, while the other variables were positively associated.

For both models, graphs of the predicted values against the observed data are shown in Figure 3. The inclusion of the seasonal component resulted in a significant reduction in the residual deviance, but plots of the autocorrelation coefficient showed that significant correlation between successive observations of human cases was still present (Figure 4). Furthermore, the estimated dispersion factors indicated that the data were overdispersed.

Discussion

The declining trends observed in the seroprevalence in slaughter-pigs and the prevalence in pork are primarily believed to be an effect of the integrated control

efforts implemented at the herd and slaughterhouse level. The programme is presumably also responsible for at least a part of the reduction observed in human *S. Typhimurium* cases. This is supported by the results of a study, which indicates that the number of human cases caused by pork-associated *Salmonella* types has been gradually reduced since 1996 (Hald and Wegener, 1999).

A seasonal pattern of the seroprevalence in Danish slaughter pigs has previously been described (Christensen and Rudemo, 1998), and may be explained by different management factors that are changing with the seasons and/or the occurrence of other diseases exhibiting a seasonal pattern. The seasonal effect may also in part be attributed to ambient temperature, as temperatures outside the pigs homeothermic range (10-20 °C) may increase the stress factor upon the pigs resulting in an excessive shedding of *Salmonella* in the environment (Warriss, 1996). A part of the seasonality observed in the prevalence of *Salmonella* in pork is probably explained by multiplication of *Salmonella* bacteria due to elevated temperatures during the summer. Proliferation of bacteria present in the abattoir environment may increase during high ambient temperatures, and thereby result in more carcasses and consequently more pork being contaminated (Hald et al., 2001). Breaches in the cool chain during processing and storage will likewise have a greater impact on the propagation of bacteria if the weather is hot.

In the glm, the number of human *S. Typhimurium* cases was found to be associated with the prevalence in pork sampled 1 to 5 weeks before case registration, whereas in the gam the association was limited to the prevalence in pork lagged at week 4 and 5. Considering that fresh pork may be sampled up till 2 weeks before it is consumed, and there may elapse 3-4 weeks from consumption till registration of the case (Figure 5), we find this association plausible. The number of cases was also found to be positively associated with the temperature 2-3 weeks before case registration in both models. Though air temperature may be a direct cause due to increased proliferation of *Salmonella* bacteria present in the pork meat, it may also be indicative for other non-measured factors. Some of these factors are probably related to changes in human behaviour in warm weather, for instance increased preparation of meat at barbecues or increased travelling activities. Studies has shown that preparing meat at barbecue increases the risk of attracting campylobacteriosis (Kapperud et al., 1992; Neimann et al., 2001). These results may to some extent also apply to human salmonellosis.

In both the glm and gam, the seroprevalence in slaughter pigs turned out to be significantly associated with the number of human cases. It is difficult to make biological meaningful inference about the seroprevalence as a predictor for human cases, especially since the strongest association appeared 15 to 35 weeks before (or after) the case was registered. One explanation could be that most new infections in

pig herds occur in the late summer resulting in high seroprevalences during the winter months.

Limitations on inferences from overall surveillance data should be recognised, as significant associations may be caused by other unmeasured factors associated with the factor measured. It may provide insight into new ways of controlling *Salmonella*, if such unmeasured factors can be elucidated. It is worthwhile to recognise and describe seasonal variations and long term trends, as it can help avoid a false impression of, for instance, the improving or declining effect of a surveillance and control programme when results from the programme are compared at different times a year or over several years. In addition, it may improve our understanding of the mechanisms behind the dynamics in the occurrence of *Salmonella* (or other zoonotic) infections in humans.

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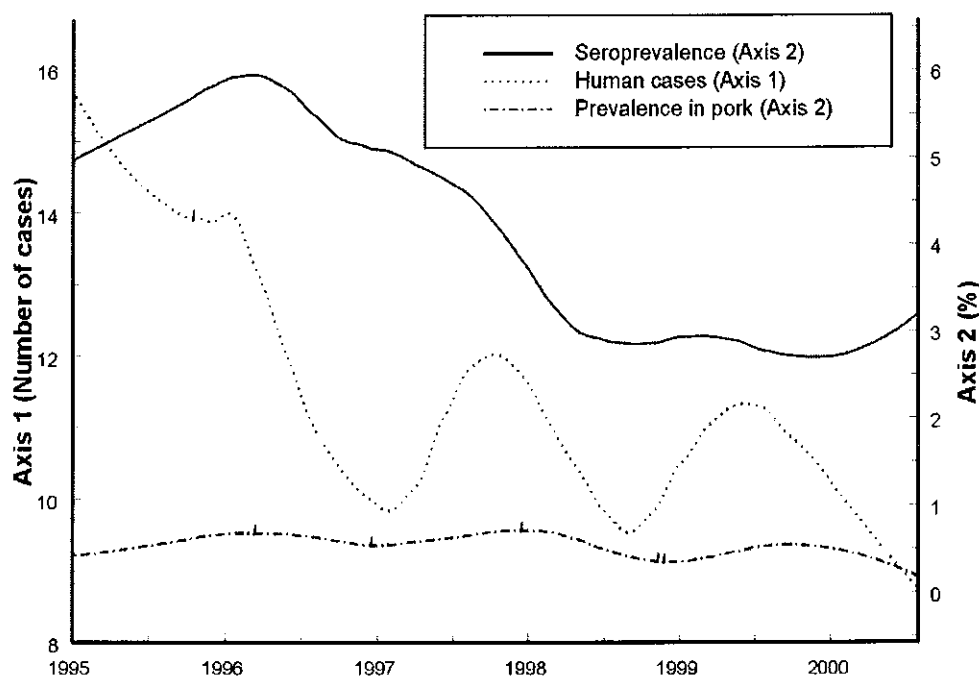


Figure 1

Trends components of the three time series: seroprevalence in slaughter pigs, prevalence of *S. Typhimurium* in pork and incidence of *S. Typhimurium* in humans.

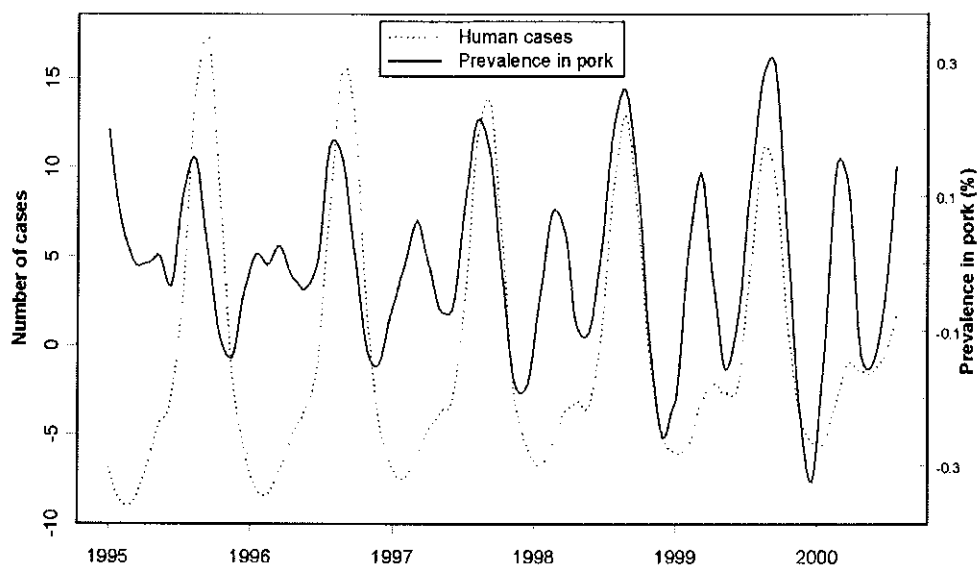


Figure 2

Seasonal components of the two time series: prevalence of *S. Typhimurium* in pork and incidence of *S. Typhimurium* in humans.

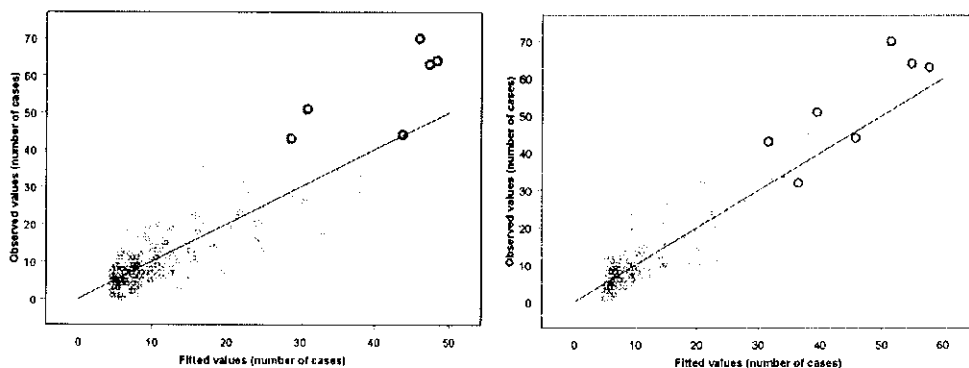


Figure 3

Observed data plotted against values fitted by glm and gam, respectively. Observations marked with big circles represent the number of cases registered during a six week long human *S. Typhimurium* outbreak in 1996.

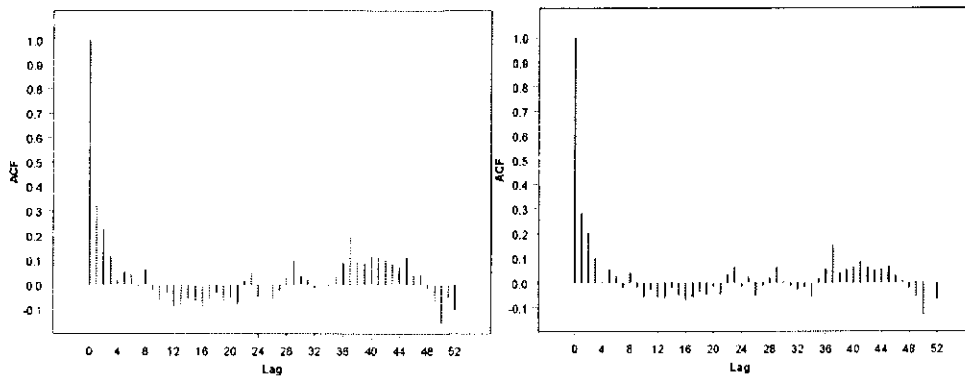


Figure 4

Plot of autocorrelation coefficients between successive observations of the number of human *S. Typhimurium* cases after fit by glm and gam, respectively.

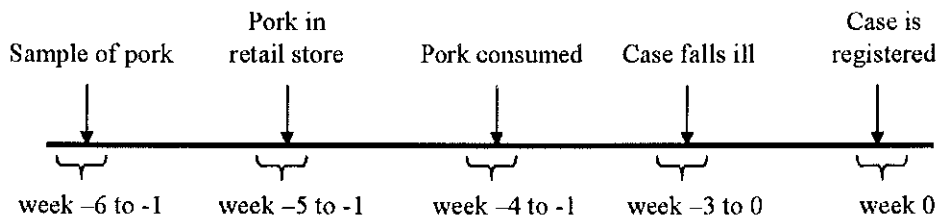


Figure 5

Illustration of the number of weeks that may elapse from the sampling of pork at slaughterhouses until a case is registered.

Table 1
Results of the final reduced general linear model

Independent variable added sequentially	Parameter estimate	Std. Error	95% Confidence Interval	Interval	Reduction in deviance	Residual deviance	P-value (χ^2)
Intercept	1.5873	0.2378	1.1191	2.0513	---	2102	---
Hours of sun week 0	-0.0016	0.0021	-0.0058	0.0026	199.77	1902	0.0000
Days with rain week 0	-0.0258	0.0207	-0.0664	0.0149	6.05	1896	0.0139
Prev. in pork week -1	0.0387	0.0510	-0.0620	0.1380	98.23	1798	0.0000
Prev. in pork week -2	0.0491	0.0497	-0.0490	0.1459	132.65	1662	0.0000
Temp. week -2	0.0178	0.0144	-0.0104	0.0459	716.13	946	0.0000
Days with rain week -2	-0.0177	0.0172	-0.0513	0.0159	0.22	946	0.6423
Prev. in pork week -3	0.0382	0.0503	-0.0611	0.1360	23.94	922	0.0000
Hours of sun week -3	-0.0007	0.0019	-0.0044	0.0030	0.49	922	0.4847
Temp. week -3	0.0169	0.0148	-0.0121	0.0459	70.37	851	0.0000
Prev. in pork week -4	0.1125	0.0491	0.0155	0.2081	37.97	813	0.0000
Prev. in pork week -5	0.0801	0.0499	-0.0185	0.1771	17.30	796	0.0000
Hours of sun week -6	0.0041	0.0025	-0.0008	0.0091	7.98	788	0.0047
Temp week -6	-0.0123	0.0112	-0.0343	0.0096	12.29	776	0.0005
Days with rain week -6	0.0318	0.0236	-0.0144	0.0782	1.22	775	0.2691
Seroprevalence	0.0720	0.0236	0.0259	0.1183	47.66	727	0.0000
Seasonal component	0.0538	0.0081	0.0379	0.0698	103.81	623	0.0000
Dispersion parameter	2.991						